Acta vet. scand. 1995, *36*, 145-147.

Brief Communication

Prostaglandin $F_{2\alpha}$ Metabolite Levels Following an Embryo Transfer Procedure in the Mare

Hormonal, chemical, and mechanical stimuli can activate the arachidonic acid cascade and result in formation of prostaglandins and related substances. These compounds can have a profound role in the initiation of the inflammatory process *(Higgins & Lees* 1984). Prostaglandin (PG) $F_{2\alpha}$ is the key hormone in reproductive physiology with well-known effects on reproductive performance e.g. luteolysis and abortion. An activation of the arachidonic acid cascade, caused by mechanical manipulation during an embryo transfer procedure, might be one explanation for early embryonic loss.

The literature is contradictory concerning the effect of uterine manipulation on the $PGF_{2\alpha}$ secretion in the pregnant mare. *Sirois et al.* (1987) found that some, but not all, mares showed a significant increase in $PGF_{2\alpha}$ secretion during non-surgical embryo transfer. On the other hand, *Bowen et al.* (1985) did not observe any changes in the mean oestrous cycle length and concluded that $PGF_{2\alpha}$ release, if present, was insufficient to induce luteolysis. However, several authors have reported that manipulation of the genital tract, i.e. cervical dilatation and/or palpation of the uterus in combination with flushing, with or without administration of pharmacologically active substances, can induce a release of $PGF_{2\alpha}$.

The aim of the present study was to investigate if there was a prostaglandin-mediated reaction in the uterus following a non-surgical embryo transfer procedure, as reflected by increased PG-metabolite levels in the general circulation.

Four Standardbred mares (A-D) with normal oestrous cyclicity were used as recipients. One of the mares (A) came into heat and ovulated naturally. The other 3 (B-D) were injected with 5 mg $PGF_{2\alpha}$ (Dinolytic® vet., Upjohn, Kalamazoo, Mich., USA) intramuscularly during the luteal phase. To induce ovulation, 3000 units of human chorionic gonadotropin (Pregnyl®, Organon, Oss, Holland) was injected intramuscularly on the second day of oestrus. Ovulations were determined by ultrasonography using a real-time linear array ultrasound scanner with a 5 MHz intrarectal probe (Aloka SSD-210, Aloka Co. Ltd, Tokyo, Japan).

A non-surgical embryo transfer method was used *(Squires et al.* 1985). A sterile 55-cm insemination catheter (diameter 4 mm) was manually guided into the cervix, a hand was then inserted into the mare's rectum to elevate the uterus. This allowed passage of the catheter through the cervix into the body of the uterus with minimal resistance. One ml of phosphate buffered saline (PBS) without an embryo was then expelled into the uterine cavity. The simulated embryo transfer procedure was performed on the 5th day (mare C), 6th day (mares B and D) and 9th day (mare A), respectively, after ovulation.

To facilitate the collection of blood, a permanent cannula was inserted into the jugular vein. Blood samples were withdrawn every 10

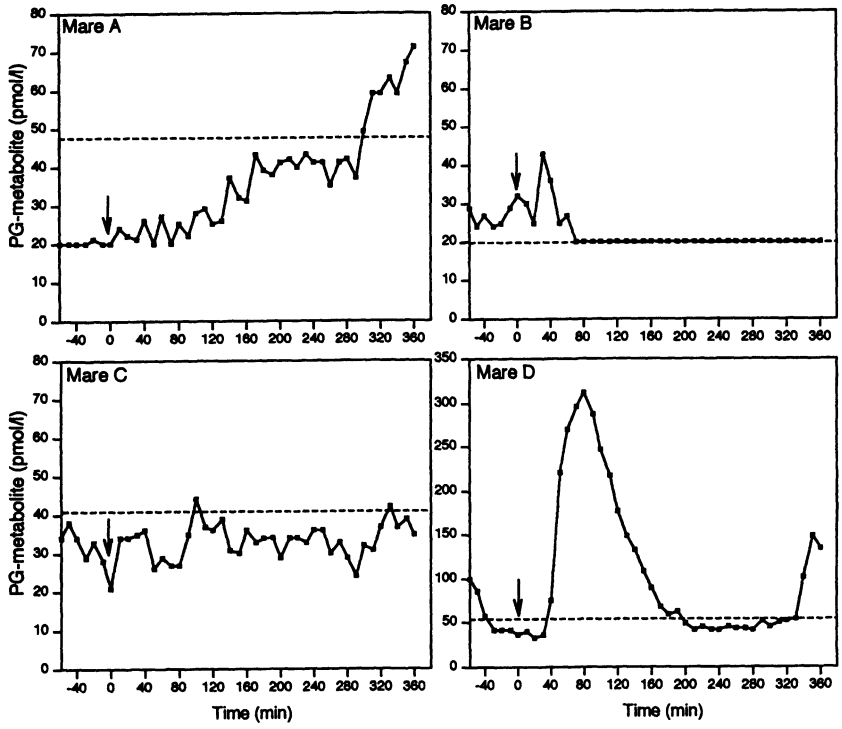

Figure 1. Levels of prostaglandin $F_{2\alpha}$ metabolite in 4 mares (A-D). The broken line indicates line of skewness and the arrow time of manipulation. Note the different scale in Mare D.

min, starting 60 min before and continuing for 6h (360 min) after the manipulation procedure. The samples were centrifuged immediately after collection and the plasma decanted and stored at –20°C until assayed.

The concentration of the main metabolite of $PGF_{2\alpha}$ was analyzed by radioimmunoassay *(Granström & Kindahl* 1982). The limit of detection is 20 pmol/l. The skewness line was calculated according to *Zarco et al.* (1984). Values greater than 2 standard deviations above the line of skewness were considered as elevated.

The cervixes of mares A and D were found to be tightly closed and consequently more difficult to pass through than in the other 2 mares. A slight tendency of an increase in the PG-metabolite level was seen in mare A with values above the skewness line 5h after the manipulation (Fig. 1). However, the oestrous interval was 21 days (mare A). A significant PG-metabolite peak appeared around 1.5h after the transfer procedure in mare D (Fig. 1). However, the prostaglandin release did not affect the oestrous interval (21 days).

The embryo transfer procedures in mares B and C were easily performed. A minor peak of the PG-metabolite was detected within the first hour after the procedure in mare B. Thereafter the detected values were under the limit of detection. In mare C, no elevated values of the PG-metabolite were recorded (Fig. 1). The oestrous intervals were 15 and 25 days in mares B and C, respectively.

After the transfer procedure, increased PG-metabolite levels were found in 2 mares, although only 1 of them had a significant peak. The cervixes of these mares were closed and consequently more difficult to penetrate than in the other 2 mares. As these manipulations required more effort, this could be one explanation for the increased PG-metabolite levels found in these animals. This is in agreement with *Roberts et al.* (1975), who reported that mechanical stimulation of the uterus caused a release of $PGF_{2\alpha}$. Even though 1 of the mares had a PG-metabolite peak near the magnitude usually associated with luteolysis, i.e. 500-1400 pmol/l *(Kindahl et al.* 1982), the corpus luteum did not regress. A single peak might not be enough to have an effect on the corpus luteum. Instead, several peaks may be required to induce luteolysis. In addition, the oestrous interval was not shortened in these 2 mares.

Despite the fact that the present study is comprised of only 4 mares, it is interesting to note that 2 of them reacted upon the manipulation with increased PG-metabolite levels. This finding suggests that a larger group of mares should be investigated to further clarify the potential for a prostaglandin-mediated reaction to a non-surgical embryo transfer procedure.

Acknowledgement

Financial support for this study was provided by the Swedish Council for Forestry and Agricultural Research.

K. Kask, L. Malmgren and K. Odensvik

Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden.

References

Bowen MJ, Salsbury JM, Bowen JM, Kraemer DC: Non-surgical embryo auto-transfer in the mare. Equine vet. J. Suppl. 3, 1985, 100-102.

Granström E, Kindahl H: Radioimmunoassay of the major plasma metabolite of $PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$. Methods Enzymol. 1982, *86,* 320-339.

Higgins AJ, Lees P: The acute inflammatory process, arachidonic acid metabolism and the mode of action of antiinflammatory drugs. Equine vet. J. 1984, *16,* 163-175.

Kindahl H, Knudsen O, Madej A, Edqvist L-E: Progesterone, prostaglandin F-2α, PMSG and oestrone sulphate during early pregnancy in the mare. J. Reprod. Fert., Suppl. 32, 1982, 353-359.

Roberts JS, Barcikowski B, Wilson L, Skarnes RC, McCracken JA: Hormonal and related factors affecting the release of prostaglandin $F_{2\alpha}$ from the uterus. J. Steroid Biochem. 1975, *6,* 1091-1097.

Sirois J, Betteridge KJ, Goff AK: PGF-2α release, progesterone secretion and conceptus growth associated with successful and unsuccessful transcervical embryo transfer and reinsertion in the mare. J. Reprod. Fert., Suppl. 35, 1987, 419-427.

Squires EL, Cook VM, Voss JL: Collection and transfer of equine embryos. Animal Reproduction Laboratory Bulletin No. 1, 1985, 38 pp.

Zarco L, Stabenfeldt GH, Kindahl H, Quirke JF, Granström E: Persistence of luteal activity in the non-pregnant ewe. Anim. Reprod. Sci., 1984, *7,* 245-267.

(Received May 26, 1994; accepted October 3, 1994).

Reprints may be requested from: K. Kask, Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, S-75007 Uppsala, Sweden.